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## **Brief Review**

### **Renal Aging. Causes and consequences**

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## **Abstract**

Individuals aged >65 years are the fastest expanding population demographic throughout the developed world. Consequently, more aged patients are receiving diagnoses of impaired renal function and 'nephrosclerosis' - age associated histological changes in the kidneys. Recent studies have shown that the aged kidney undergoes a range of structural changes and has altered transcriptomic, haemodynamic and physiologic behaviour at rest and in response to renal insults. These changes impair the ability of the kidney to withstand and recover from injury, contributing to the high susceptibility of the aged population to acute kidney injury, and their increased propensity to develop subsequent progressive chronic kidney disease. This review examines these features of the aged kidney, and explores the various proven and putative pathways contributing to the changes seen with aging in both experimental animal models and in man. The potential for further study to increase understanding of the aged kidney, and to lead to novel therapeutic strategies is discussed.

## **Introduction**

The Centre for Disease Control predicts that 72 million Americans will be aged 65 years or older by 2030, accounting for approximately 20% of the U.S. population.<sup>1</sup> Eurostat predicts that 28% of Europeans will be aged over 65 by 2060.<sup>2</sup> These increasing numbers of elderly individuals will inevitably lead to increasing diagnoses of age related kidney impairment.

In renal aging a complex interplay of genetics, environmental change and cellular dysfunction leads to characteristic structural and functional changes.<sup>3</sup> This review summarises our current understanding of the factors driving age-associated changes in the kidney.

## **Clinical features of renal aging in man**

### ***Structural changes of aging***

With age there is a decline in total nephron size and number, tubulointerstitial changes, glomerular basement membrane thickening and increased glomerulosclerosis (Figure 1).<sup>4,5</sup> This age-related histological appearance is frequently described as “nephrosclerosis” and describes a combination of 2 or more histological features: any global glomerulosclerosis, tubular atrophy, interstitial fibrosis > 5% and any arteriosclerosis. A study of healthy kidney donors demonstrated nephrosclerosis in only 2.7% of biopsies from donors aged less than 30 years, 58% from 60-69 year olds and 73% from donors

aged greater than 70.<sup>6</sup> Cadaver studies estimate that the upper limit of normal glomerulosclerosis in aging exceeds 10%.<sup>7</sup>

Nephrosclerosis remains a poorly understood observation, and its importance within an aging kidney is far from clear. We know that nephrosclerosis correlates with aging and mild hypertension in healthy living donor kidneys.<sup>8</sup> Importantly however, age related decline in measured GFR does not correlate with the presence or absence of nephrosclerosis.<sup>9</sup> In fact, nephrosclerosis does not correlated with urine albumin excretion, family history of end-stage renal disease, body mass index, serum cholesterol, glucose, or uric acid.<sup>10</sup> It remains unclear then, whether nephrosclerotic changes have any contribution to the functional changes seen in aging, or are perhaps distinct and unrelated.<sup>11</sup>

### ***The Aging-CKD spectrum***

Our understanding of the pathways underlying renal aging is incomplete and derived from studies of healthy aging kidneys and extrapolation from experimental and clinical studies of CKD.

It is important to note the distinction between these conditions, with the mechanisms of progressive genetic, immune or toxin mediated injury seen in CKD distinct from the gradual, prevalent changes seen in the aging kidney. Throughout this review we will focus on the changes seen in the ‘healthy’ aged kidney, though due to the paucity of experimental and clinical data

available in aging kidneys at times reference will be made to mechanisms in progressive CKD which may also be of relevance to the uninjured but aged kidney. Processes discussed below such as cellular senescence, fibrosis, vascular rarefaction and glomerular loss are common to both aging and CKD despite differences in causation and natural history. Similarities are also seen in the behaviour of the chronically damaged and the aged kidney including their heightened susceptibility to further injury and deficient repair.<sup>13</sup>

### ***Declining Glomerular Filtration Rate (GFR)***

Population GFR declines with age with longitudinal studies differing in their reported rates of decline.<sup>14,15</sup> While the MDRD study suggested renal function declined at a rate of 3.8ml/min/year/1.73m<sup>2</sup>, rates as low as 0.4 ml/min/year/1.73m<sup>2</sup> in the Netherlands have been described.<sup>16–19</sup> A Japanese cohort study suggests the rate of GFR decline increases with advancing age.<sup>20</sup>

Studies of robustly phenotyped Kuna Indians with minimal prevalence of hypertension and cardiovascular disease demonstrate comparable declines in renal function over time, suggesting that there is a true age related decline, rather than the cumulative effects of cardiovascular disease.<sup>21</sup> How a significant minority of individuals apparently remain free of nephrosclerosis and GFR loss remains poorly understood and merits further study.

### ***Decreased Tubular Function***

Aging is characterised by progressive tubular dysfunction, decreased sodium reabsorption, potassium excretion and urine concentrating capacity potentially contributing to an increased susceptibility to AKI.<sup>22–24</sup> Elderly patients demonstrate decreased trans-tubular potassium gradients and fail to increase distal tubule potassium excretion when hyperkalaemic or in response to fludrocortisone.<sup>25</sup> Decreased potassium excretion correlates with decreasing GFR, and may reflect a degree of reduced sodium and chloride delivery to the distal convoluted tubule.<sup>26</sup>

### ***Vascular Changes***

There are important changes to blood vessel structure and function in the aging kidney. There is increased extracellular matrix (ECM) deposition, increased intimal cell proliferation in pre-glomerular arterioles and increased intrarenal shunting and capillary bypassing predominantly affecting the cortex.<sup>27</sup>

Increased renal sympathetic tone increases vasoconstriction whilst aortic baroreceptor attenuation of sympathetic tone decreases with age.<sup>28,29</sup> Renal vasodilators such as atrial natriuretic peptide, nitric oxide (NO) and amino acids become less effective.<sup>30–32</sup> Human studies demonstrate decreased NO production and platelet responsiveness,<sup>33</sup> with accumulation of the NO synthase inhibitor asymmetric dimethylarginine in elderly individuals.<sup>34</sup> In particular, aging males become increasingly NO dependent to maintain renal plasma flow.<sup>35</sup>

## Biological Processes and Mediators Implicated in Experimental Aging

Most rodent experimental models of renal disease are undertaken in young animals, potentially affecting their relevance to the aging kidney. There is limited or no data available regarding the response of the aged rodent kidney to experimental glomerulonephritis, AKI, ureteric obstruction, diabetic nephropathy, 5/6<sup>th</sup> nephrectomy, adriamycin nephropathy or renal transplantation. Some aspects of renal aging may be studied *in vitro* but others require study *in vivo* in aged mice or other experimental animals (Table 1).

Studies have demonstrated increased susceptibility of the aged kidney to ischemia reperfusion injury (IRI) or toxic AKI.<sup>36,37</sup> Aged mice exhibit increased mortality, AKI severity and chemokine/cytokine responses in a model of uterine sepsis.<sup>38</sup> Furthermore, aged mice exhibited increased mortality, prolonged injury, reduced regeneration, increased scarring and microvascular rarefaction following renal IRI compared to young mice.<sup>39</sup>

The biology of aging is complex involving diverse changes to cells, tissues, organs and the surrounding microenvironment (Figure 2). Many of these processes and mediators are discussed below but the reader should appreciate that this list is not exhaustive.



## **A) Signalling pathways and oxidative stress in the aging kidney**

### ***Falling Klotho levels***

Klotho is a transmembrane protein strongly expressed in the kidney and a co-receptor for fibroblast growth factor-23 (FGF-23). Whilst its exact physiological role in aging remains incompletely understood Klotho has a role in modulating diverse aging associated pathways. These include calcium and phosphate metabolism with implications for vascular calcification, hypoxia, cellular regeneration and senescence. Indeed, homozygous transgenic Klotho knockout mice demonstrate arteriosclerosis and vascular changes as part of their aging phenotype.<sup>40</sup> Similarly, FGF-23 knockout mice display high serum phosphate and increased renal phosphate reabsorption in addition to their aging like phenotypes.<sup>41,42</sup> It may be that these vascular changes contribute directly to the aging phenotype we observe.

Klotho's effects on tissue function, autophagy and fibrosis could contribute to abnormal healing and possibly nephrosclerosis.<sup>43,44</sup> Importantly, Klotho deficient mice exhibit reduced lifespan, skin and muscle atrophy, osteoporosis and ectopic calcification.<sup>45</sup> Conversely, mice overexpressing Klotho have a longer mean lifespan.<sup>43</sup>

Klotho decreases epithelial senescence in response to oxidative stress, reduces binding of nuclear factor kappa-B (NF $\kappa$ B) and increases cell survival

in experimental uremia.<sup>46</sup> Klotho also represses insulin and insulin-like growth factor 1 (IGF1) signalling, likely contributing to reduced oxidative stress in mice and *in vitro* models employing Klotho overexpression.<sup>43,45,47</sup> Importantly, Klotho supplementation in a rat UUO model attenuated renal fibrosis.<sup>48</sup>

### ***Increasing Wnt Activation***

Mechanisms for the anti-fibrotic effects of Klotho include suppression of fibroblast growth factor and modulation of Wnt signalling.<sup>49–51</sup> Wnt is a conserved signalling pathway activated post injury which promotes pro-fibrotic gene expression.<sup>52</sup> As Klotho levels fall during aging Wnt signalling increases promoting fibrosis and vascular calcification<sup>53</sup> though further experiments are required to clarify causality. Wnt activation promotes renal fibrosis in murine models and is a target for inhibition<sup>54,55</sup> with antagonism of Wnt and its downstream targets ameliorating experimental renal fibrosis.<sup>56,57</sup> The interplay between potentially causative pathways is illustrated by studies demonstrating that renin-angiotensin-aldosterone signalling is Wnt mediated with experimental blockade protecting mice from post-injury fibrosis and proteinuria.<sup>58</sup>

### ***Declining Peroxisome Proliferator-activated Receptor gamma (PPAR $\gamma$ ) levels***

PPAR $\gamma$  is a nuclear receptor whose activity decreases with age in experimental rodent models, whilst PPAR $\gamma$  agonists increase Klotho expression.<sup>59,60</sup> The PPAR $\gamma$  pathway protects against oxidative stress and improves vascular function *in vitro* and in aging rats<sup>61–63</sup> with PPAR $\gamma$  agonists

protecting human fibroblasts against features of aging and oxidative stress *in vitro*.<sup>64</sup> PPAR $\gamma$  agonism by pioglitazone or baicalin improves age related vascular oxidative stress or renal inflammation respectively, providing a potential therapeutic strategy for elderly patients with reduced PPAR $\gamma$  activity.<sup>60,65</sup>

### ***Angiotensin II***

Angiotensin II (AT2) is increased in aged rats compared to young controls,<sup>66</sup> driving increased fibrosis, glomerular cell growth and ECM accumulation,<sup>67</sup> altered mitochondrial redox function and cytoplasmic oxidative stress in the aging kidney.<sup>66,68,69</sup> Angiotensin I receptor activation simulates the pro-fibrotic  $\beta$ -catenin/Wnt pathway mentioned above.<sup>70</sup> Treating aging rats with captopril reduces TGF- $\beta$  activity and attenuates renal fibrosis.<sup>71,72</sup> AT2 antagonism via ACEi/ARB improves mitochondrial number and function in rats and further studies are warranted.<sup>73</sup>

### ***Oxidative Stress***

A balance exists in tissues between reactive oxygen species(ROS) generation and oxidant scavenging and defence mechanisms. When this balance is disturbed, either by increased generation of ROS, decreased detoxification or both, then oxidative stress may occur. It has been hypothesised that oxidative stress leads to tissue damage and contributes to the aging phenotype. Certainly, there is evidence in murine and human studies, of both increased ROS generation and altered oxidant removal in aging.<sup>74–76</sup>

There is a continuous generation of oxidative species through various mechanisms, including mitochondrial oxidative phosphorylation, which increases within the aging kidney.<sup>76,77</sup> Studies in aged rat kidneys support the theory there is also reduced oxidant defence demonstrating decreased antioxidative capacity and reduced levels of Cu/Zn-SOD, catalase and GSH reductase.<sup>78,79</sup> This overall increased oxidative load may contribute to chronic cellular stress and mitochondrial injury<sup>77</sup> as well as apoptosis and possibly inducing tubular cell damage.<sup>80,81</sup>

Contributing to this increased oxidative stress, it has been noted that sirtuins (important antioxidant molecules) are diminished with age. Sirtuins protect against renal inflammation, fibrosis and apoptosis while improving autophagy.<sup>82,83</sup> Thus, defective ability to respond to cell stress in aged kidneys may contribute to the aged phenotype.<sup>84</sup> Mouse models of reduced SIRT-1 expression demonstrate increased apoptosis and fibrosis following UUO.<sup>85</sup> Additional Sirtuin functions include histone deacetylation and regulation of transcription factors controlling cellular stress and survival.<sup>86,87</sup> Altered Sirtuin levels in aging may contribute to aging phenotypes by altering the kidneys capacity to respond to oxidative stress and thus suffer increased oxidative DNA damage.<sup>88,89</sup> Interestingly, angiotensin-II (AT2) downregulates SIRT-3 *in vitro*, suggesting that the damaging effects of raised AT2 levels and low Sirtuin levels may be related in the aging kidney.<sup>90</sup>

## **B) Cell cycle progression in the aged kidney**

Aged animals have reduced proliferative responses after experimental IRI. Tubular epithelial cells in aged mice express higher levels of zinc-alpha (2)-glycoprotein (AZGP1), limiting proliferation following IRI.<sup>91</sup> Whilst reduced proliferation might be expected to delay recovery, AZGP1 knockout mice displayed worsened fibrosis after IRI with AZGP1 administration being protective, implicating control of proliferation as a mechanism limiting fibrosis with aging.<sup>92</sup> Studies in several CKD models demonstrate G2/M arrest in tubular epithelial cells promotes renal fibrosis, but no studies have examined G2/M arrest in aging kidneys.<sup>93</sup>

Cellular senescence, defined as a state of permanent cell cycle arrest, is a key anti-proliferative response to aging and injury. This crucial process shuts down damaged cells, protects against malignant transformation and limits excess fibrosis at both baseline and following injury.<sup>94</sup>

Senescence may occur as a result of repeated cell division and telomere shortening ('replicative senescence') or following factors such as oxidative stress or genotoxic injury ('stress induced premature senescence' [SIPS]) (Figure 3).<sup>95</sup> Increased numbers of senescent cells accumulate in multiple organs including the kidney with advancing age (identified by p16<sup>INK4a</sup> or senescence-associated  $\beta$ -galactosidase expression).

Cell senescence limits fibroblast proliferation in tissue wounds however there is increasing interest in the role of the Senescence Associated Secretory Phenotype (SASP) in promoting fibrosis.<sup>94</sup> SASP promotes fibrosis and organ

dysfunction in aging via release of factors including Interleukins-6 and 8, Wnt16B and GRO $\alpha$ .<sup>96–98</sup> Studies in murine renal transplantation showed that renal p16<sup>INK4a</sup> deletion reduced pathologic changes and interstitial fibrosis post ischemia reperfusion injury, supporting clinical findings that cellular senescence contributes to adverse long-term allograft outcomes.<sup>99</sup> Cell stress is known to induce SIPS, and consistent with this porcine models have shown that renal p16<sup>INK4a</sup> expression increases after IRI.<sup>100</sup> Interestingly, p16<sup>INK4a</sup> knockout mice exposed to experimental renal injury show improved recovery after IRI but worsened fibrosis after UUO.<sup>101,102</sup> These superficially inconsistent findings may reflect the different pathological processes at play, with p16<sup>INK4a</sup> deficiency leading to less cell death and enhanced regenerative proliferation in AKI, but the lack of p16<sup>INK4a</sup> induced senescence inducing an exaggerated, maladaptive fibroblast response to ongoing injury in UUO.

Recent seminal studies used transgenic animals to induce specific depletion of p16<sup>INK4a</sup> expressing senescent cells and demonstrated reduced markers of aging in multiple organs including the kidney and increased overall lifespan.<sup>103</sup> Other work has used Bcl2/xL inhibitors to deplete senescent cells in non-transgenic animals.<sup>104</sup> Whilst these findings open up exciting new therapeutic avenues for the selective targeting of senescent cells to prolong healthy lifespan, further studies focusing upon the aging kidney required.

### ***Telomere Shortening***

Telomeres are nucleotide sequences which act as a defensive “cap”: limiting activation of DNA repair pathways, protecting genetic material and minimising

background cellular stress response.<sup>105,106</sup> Although telomere length declines with age, it remains controversial whether this is a primary process or a by-product of aging.<sup>105,107</sup> As telomeres shorten with aging and oxidative stress, chromosome instability ensues, leading to cellular instability, senescence and subsequent apoptosis.<sup>108</sup>

Increased telomere shortening in telomerase deficient mice is associated with increased tubular injury and reduced tubular proliferation after renal IRI with reduced tubular cell autophagy implicated in the limited regenerative response.<sup>109,110</sup> This implies a potential causal role for telomere shortening in some of the vulnerability of aging kidneys to injury and it is noteworthy that experimental elongation of shortened telomeres resulted in partial reversal of aged organ degeneration.<sup>111</sup>

### **C) Hypoxic Damage and Disordered Repair.**

Under physiological conditions, the kidney is supported by a network of resident mononuclear phagocytes and pericytes contributing to tissue homeostasis and vascular stability. Renal oxygen delivery and the functional status of resident and recruited cells in the kidney have been shown to alter in aged and injured experimental animals.

#### ***Hypoxia***

Whilst the healthy kidney has areas of low oxygen tension, reduced capillary density and increased hypoxia is recognised as a potential driver of CKD, and its role in normal aging is being explored. In experimental CKD, the expected

angiogenic response to hypoxia fails, instead resulting in fibrosis.<sup>112</sup> Increased renal hypoxia has also been demonstrated throughout aged rat kidneys, most prominently in the cortical zones, as detected by use of the hypoxia sensitive marker pimonidazole.<sup>113</sup> Aged rat kidneys demonstrate decreased VEGF globally and increased anti-angiogenic thrombospondin-1, resulting in capillary loss with increased glomerular sclerosis.<sup>114</sup> Recently reported techniques to quantify subtle changes in the renal vasculature have potential to yield new information on the evolution of renal circulatory changes and hypoxia with advancing age.<sup>115</sup>

### ***Leukocytes***

Changes in leukocyte function promoting inflammatory activation occur with aging, though whether this is a cause or effect of aging remains unclear.<sup>116</sup> Increased inflammatory signalling and macrophage infiltration,<sup>117</sup> with alterations in inflammasome components such as NOD-like receptor P3 (NLRP3), NLRC4, pro-caspase-1, NF $\kappa$ B and cytokines including IL-1 $\beta$  and IL-18 occur in aging.<sup>118</sup> Aged murine macrophages demonstrate impaired autophagy and reduced nitrate release and phagocytosis.<sup>119</sup> Healthy aged mice have increased glomerular macrophage numbers with increased macrophage infiltration evident post injury, with renal IRI models showing an increased influx of macrophage and T lymphocytes.<sup>39,120</sup> Additionally, aged mice show defective upregulation of the cytoprotective enzyme hemoxygenase-1 after IRI, with pharmacological macrophage hemoxygenase-1 induction protecting against subsequent IRI.<sup>36</sup> Finally, aged macrophages express reduced anti-inflammatory IL-10 during tissue repair in



non-renal injury models.<sup>121</sup> Given the importance of IL-10, and the negative prognostic role of macrophage infiltrates in human renal disease, these aging-associated changes potentially contribute to the increased rates of injury and maladaptive repair seen in aged kidneys.

Further evidence for the importance of the aging immune system in renal aging comes from young-old bone marrow transplant (BMT) studies demonstrating that aged animals receiving BMTs from young mice exhibited reduced renal fibrosis and cellular senescence.<sup>122</sup>

### ***Pericytes***

Although important for microvascular health pericytes are also recognised as key cells in renal fibrosis.<sup>123,124</sup> In aged mice renal pericytes decline in number and adopt a pro-fibrotic phenotype,<sup>125</sup> implicating them in aging related fibrotic changes. Pericyte-endothelial detachment under pathological conditions and their differentiation into myofibroblasts promotes microvascular rarefaction, hypoxia and fibrosis.<sup>126,127</sup> Proposed mediators of this pericyte-endothelial cross talk include VEGF and PDGF<sup>128</sup> and blocking this pericyte-endothelial interaction attenuates microvascular damage and interstitial fibrosis.<sup>129,130</sup>

### ***Disordered Repair.***

The normal enzymatic equilibrium is disturbed in aging and the balance of metalloproteinases (MMP) shifts towards fibrosis potentially via upregulation of tissue inhibitor of metalloproteinase-1 and increased leukocyte recruitment,<sup>51</sup> a pattern likely to result in increased collagen deposition.

Longitudinal studies of aging mice show increased Collagen I, III and TGF- $\beta$ 1<sup>51</sup> whilst aging rat kidneys exhibit increased ECM deposition and TGF- $\beta$ 3 expression and decreased MMP1 activity suggesting altered collagen production and processing.<sup>131</sup> Further non-inflammatory pathways may contribute to histological changes seen, including pathways driven by Wnt and AT2 as mentioned.<sup>55</sup>

## **The Aging Human Kidney**

The clinical implications of renal aging in man extend beyond changes in glomerular and tubular function. Although data generated by animal studies implicate multiple pathways of potential importance for human renal aging (Figure 4), data supporting their involvement in man is currently sparse, with further studies required.

### **A) Signalling pathways and oxidative stress in the aging kidney**

#### ***Falling Klotho levels***

Klotho and FGF-23 are present in human kidneys.<sup>132</sup> Klotho levels decline with age, and are implicated in accelerated age-related CKD and atherosclerosis.<sup>133,134</sup> Conversely, patients with increased functional Klotho expression are reported to have increased lifespan.<sup>135</sup> As Klotho falls, FGF-23 levels increase, and alter phosphate and calcium homeostasis. Clinical studies in dialysis and CKD patients show that higher FGF-23 levels associate with increased mortality.<sup>136</sup>

### ***Increasing Wnt activation***

Whilst direct evidence of Wnt activation in human aging is lacking, several Wnt antagonists are now undergoing Phase I clinical trials for cancer therapy in man.<sup>137</sup> If effective, these agents offer new therapeutic options for aging associated or fibrotic renal disease.

### ***Declining PPAR $\gamma$ levels***

Agonists of PPAR $\gamma$  are used clinically as anti-diabetic agents. Retrospective reviews of renal outcomes in clinical practice suggest that augmented PPAR $\gamma$  activity opposes proteinuria in these patients.<sup>138</sup> A meta-analysis of PPAR $\gamma$  use has also demonstrated that they associate with reduced rates of cerebrovascular disease, supporting a role in delaying age-associated pathology.<sup>139</sup> There is a need for prospective trials assessing their effects on renal function.

### ***Angiotensin II***

Despite decreased plasma renin activity in the elderly serum angiotensin II levels do not fall and hypersensitivity to angiotensin II develops in the renal vasculature.<sup>140,141</sup> Whilst ACEi and ARB drugs are in widespread use, there is a lack of human data on the impact of AT2 blocking treatments on normal renal aging and outcomes at present.

### **Oxidative Stress**

As discussed, oxidative stress represents a disruption of the balance of oxidant handling in tissues. In man longitudinal studies demonstrate increased oxidative stress in normal aging and CKD.<sup>74,142</sup> Research has focused on advanced glycation end products as drivers of oxidative stress in aging. These molecules accumulate with age and are associated with increased arterial stiffness, inflammation, oxidative stress and declining renal function.<sup>143</sup> One pharmacological attempt to modify anti-oxidant status in patients with diabetic nephropathy showed no impact on proteinuria despite increased circulating antioxidant levels.<sup>144</sup> Whether an alternative, longer term treatment approach in the healthy aged population might have efficacy remains untested.

## **B) Cell cycle progression in the aged kidney**

The presence of increased numbers of senescent cells has been noted in chronic allograft nephropathy and have been proposed as drivers of the progressive fibrosis seen.<sup>145</sup> Recent advances in our understanding of the roles of aging and stress in inducing the detrimental SASP phenotype adds to the importance of senescence cells found in both aged and disease affected human renal biopsies.<sup>146–148</sup> In humans, senescence is maximal in the medulla, potentially reflecting increased oxidative and cellular stress and relative hypoxia resulting from the vascular changes discussed previously.<sup>149</sup>

## ***Telomeres***

Telomeres shorten in human kidneys at a rate of 0.25% length per year.<sup>150</sup> While telomere shortening provides an elegant explanation of cellular aging, currently no data exists to link shorter telomeres to any histological or functional measure of renal aging. Shorter telomeres associate with CKD and worse cardiovascular outcomes and are shorter in diabetic nephropathy where they associate with rates of disease progression.<sup>151,152</sup> Furthermore, studies of hemodialysis patients show increased rates of telomere attrition suggesting they shorten in response to the physiological stress.<sup>153</sup> Although intriguing, the importance of telomere shortening in human aging remains to be elucidated.

### **C) Hypoxia, inflammation and nephrosclerosis in the aged kidney**

Due to the inherent risks of renal biopsy, samples of healthy aged kidney are seldom available for assessment of levels of nephrosclerosis, and there are no time course studies available to chart the temporal relationships of the histological findings in the aged kidney. Ongoing progress in imaging technology should enable serial non-invasive assessment of renal perfusion, vascular resistance, hypoxia, inflammation and atrophy in healthy young and aged volunteers.

#### ***Renal Hypoxia***

The clinical use of BOLD MRI imaging has demonstrated a lower pO<sub>2</sub> in older kidneys compared to younger subjects.<sup>154</sup> As intrarenal vascular disease contributes to increased glomerular sclerosis in aged biopsies it is possible

that subclinical disease leads to hypoxia before marked macroscopic changes occur.<sup>155</sup>

### ***Inflammation***

Inflammation is increased within the aging kidney in man, with pro-inflammatory cytokines detectable in the serum correlating with age related renal disease.<sup>156,157</sup>

### ***Future Research***

Reviewing the current evidence base in clinical and experimental renal aging it is clear that more work is required to understand which pathways are dispensable and which represent 'master regulators' of the aging phenotype. Studies in aged animals should allow characterisation of both the importance and interdependence of factors predisposing aged kidneys to injury, fibrosis and maladaptive repair, with subsequent validation in man. Due to the time and cost constraints inherent in using aged animals, establishing whether models of genetically accelerated aging such as the BubR1 progeroid mouse represent useful models of renal aging will be of value.<sup>158</sup> BubR1 mice have a shortened lifespan and exhibit a variety of age related phenotypes, including sarcopenia, cataracts, fat loss, cardiac arrhythmias, arterial wall stiffening and impaired wound healing. Specific to kidney research, BubR1 deficient mice also demonstrate higher senescence-associated beta-galactosidase activity in kidney sections than aged matched controls.<sup>159</sup> Whether they truly manifest a renal aging phenotype is yet to be determined.

### ***Circulating factors***

Heterochronic parabiosis with aged and young mice sharing a common circulation has provided evidence in non-renal models that circulating factors may modulate features of aging including impaired regeneration and increased fibrosis.<sup>160–162</sup> Proposed factors include  $\beta$ 2-microglobulin and growth differentiation factor 11 and reversal of changes in the brain, cardiac and skeletal muscle has been shown.<sup>163–165</sup> Debate continues as to the significance of individual factors.<sup>166–170</sup> Whether such factors impact the function of the aged kidney remains completely unknown.

### ***Novel experimental species***

Undertaking studies of experimental renal disease in aged mice is challenging and other organisms may be of use. Zebrafish have been used as a model for AKI and nephron regeneration and exhibit aging associated abnormalities.<sup>171–173</sup> Thus the use of genetically manipulated zebrafish in renal aging studies may be informative.

### ***Novel therapeutic strategies***

Many pathways implicated in the aging process are the target of interventions to improve the aging phenotype in experimental mice (Figure 5). Klotho agonists are under investigation via repurposing of established agents including PPAR $\gamma$  agonists, ACEI and ARB drugs. The importance of maintaining a normal renal microvasculature and pericyte pool is increasingly understood<sup>174</sup> and developing strategies to quantify microvascular function and to promote endothelial and pericyte health is a pressing clinical need.<sup>115</sup>

Drugs targeting cellular senescence ('senolytics') include siRNA therapies, the experimental agent navitoclax and the licenced drugs dasatinib and quercetin.<sup>175</sup> In experiments these agents demonstrate selective toxicity to senescent cells, and their potential utility in animal models and man merits further study.

### **Genetics**

Genome wide association studies (GWAS) have identified upregulation of several genes with aging. Whilst cumulative damage may well influence much of the elderly genetic milieu, candidate genes have declared themselves as being consistently highly expressed in aged kidneys.<sup>176–178</sup> Despite the utility of GWAS in identifying disease specific pathways, it has proved difficult to discover any canonical aging pathways with GWAS.<sup>179</sup>

The most promising genes encode for modulators of the glomerular filtration barrier, fibrosis and inflammatory mediators although difficulty arises when identified candidate genes do not match the experimental observations or models.<sup>180,181</sup> Transcriptomic analysis identified 427 genes strongly associated with renal aging, including mortalin-2, a heat shock protein which may counteract cell senescence and IGF receptor, a target of Klotho.<sup>182–184</sup>

GWAS remains however, a promising tool as whole genome analyses of GWAS data suggest that over 80 % of the heritability of aging is explained by common genetic variants.<sup>185</sup> Future GWAS will continue to generate



meaningful results as more advanced statistical techniques develop, and researchers increase statistical power by increasing samples number, combining studies using meta-analytical techniques, multicentre collaborations and including more extreme phenotypes in the data.<sup>185–188</sup>

### ***Epigenetics***

Epigenetics is the study of genome changes that do not alter DNA sequence. Epigenetic changes in aging include methylation and deacetylation of histone lysine residues, chromatin changes and increased transcriptional noise.<sup>179,189</sup> Interestingly, similar changes in DNA methylation and histones are associated with CKD disease progression.<sup>190–192</sup> The role of microRNA expression in modifying gene expression and nephrosclerosis is of interest,<sup>193</sup> with data in other organs suggesting an influence on aging.<sup>194</sup>

### **Conclusion**

Renal aging is complex and remains incompletely understood. Decreased protective factors, hypoxia and microenvironmental stress drive increasingly disordered inflammation and renal fibrosis. The resulting fibrosis, senescence and microvascular rarefaction exacerbate damage and promote progression. The future of treating renal aging likely lies in understanding the key initiating events and the common downstream pathways present in kidney aging that may be shared with CKD. This knowledge should allow the development of therapies capable of arresting the key mechanisms early to preserve kidney function throughout life.

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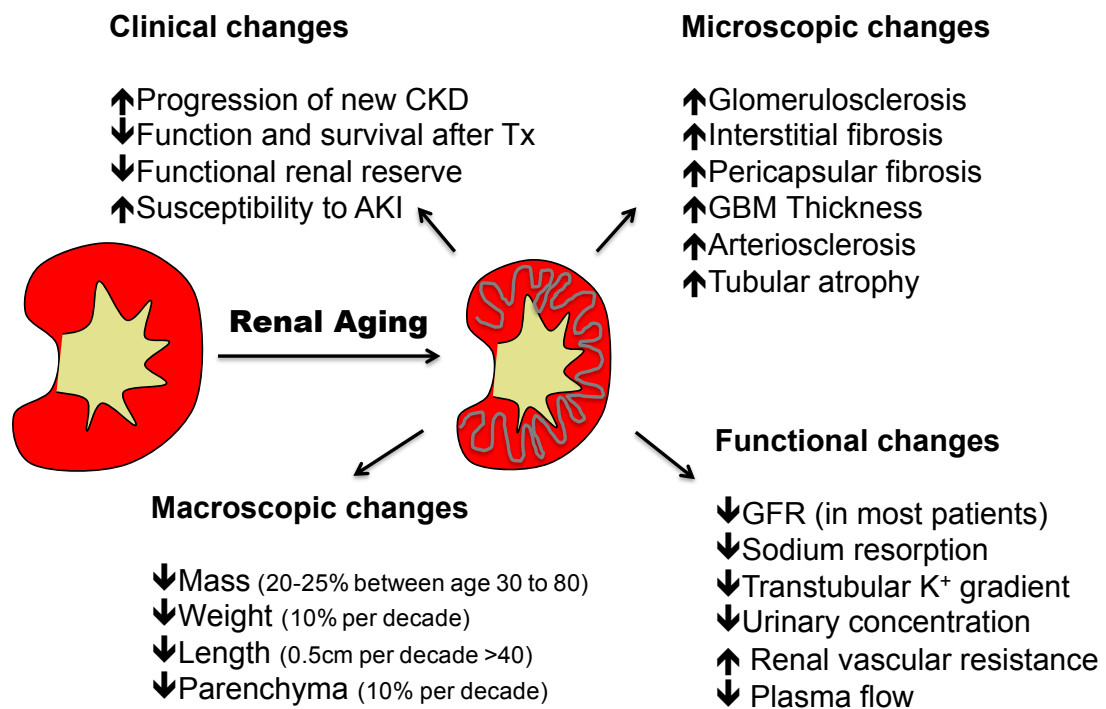
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## Figure 1 - Functional and structural changes in the aging kidney

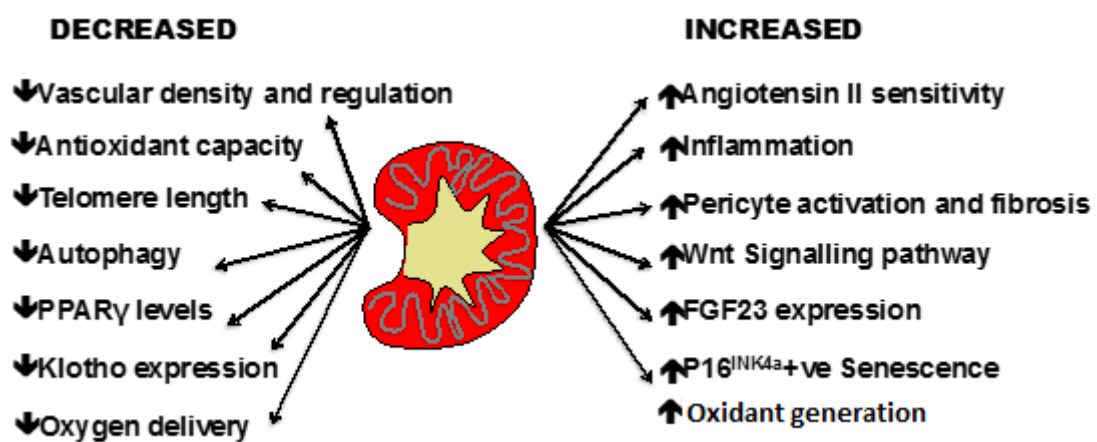
With increasing age there are alterations in the function of the kidney. These are accompanied by both macroscopic and microscopic changes and result in an alteration in the response of the kidney to diverse insults.





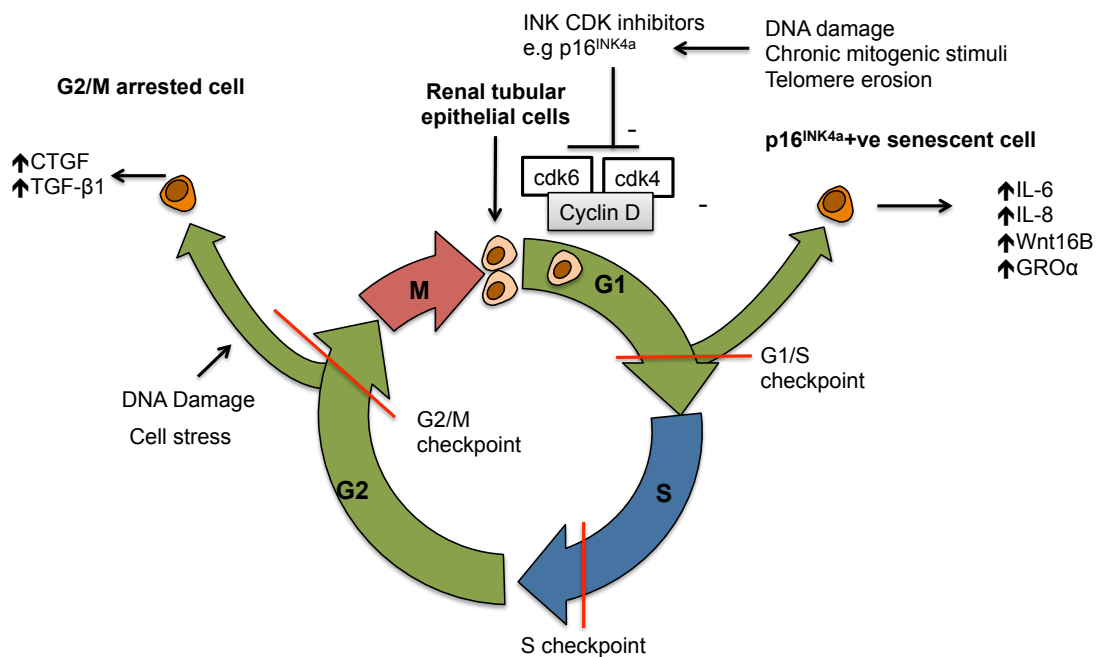
**Figure 2 – Alterations in cellular and physiological pathways in the aging kidney**

Diverse physiological, cellular and gene expression alterations occur in the aging kidney, impacting on homeostasis, function and the response to renal injury



### Figure 3 - Cell cycle progression in the aged kidney

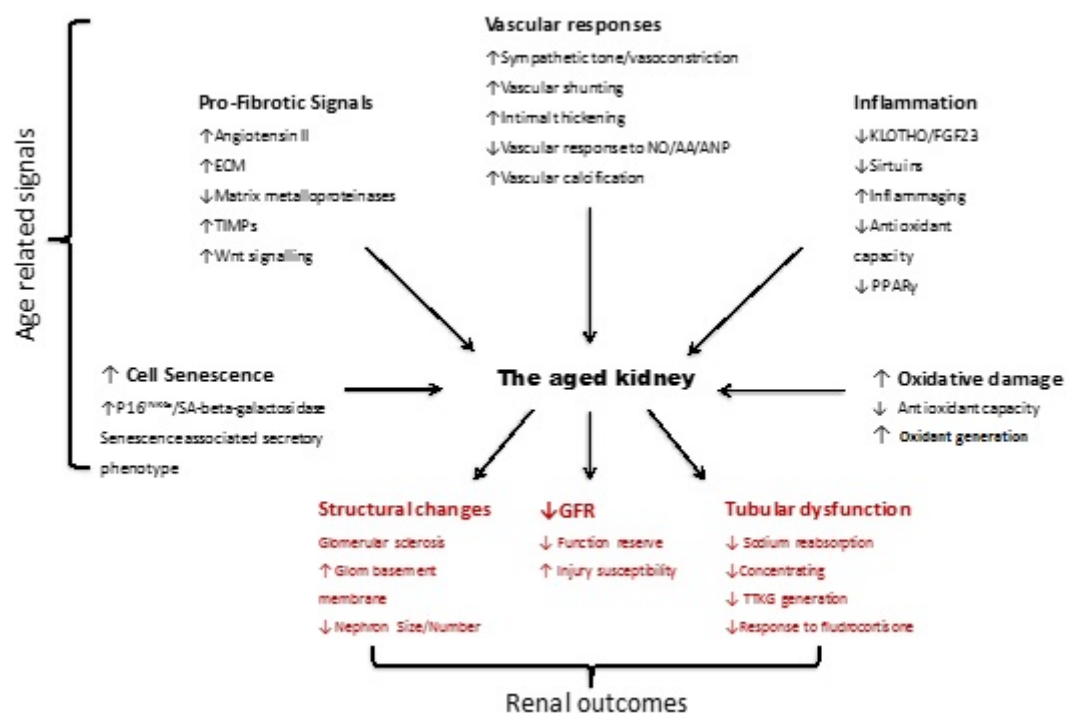
Cell cycle arrest in G1/S phase becomes more prevalent with age and results in p16<sup>INK4a</sup> positive senescent cells expressing multiple cytokines promoting autocrine and paracrine changes in aged kidneys. Whilst studies are lacking in aged animals, increased G2/M cell-cycle arrest in response to injury promotes maladaptive repair in murine kidney injury with raised G2/M counts correlating with fibrosis.<sup>93,195</sup> G2/M cell-cycle arrest may have variable effects in different cell types, being profibrotic in renal tubular cells, but preventing intimal hyperplasia in young smooth muscle cells.<sup>196</sup>



**Figure 4 - Age related pathways contributing to altered renal outcomes in the elderly**

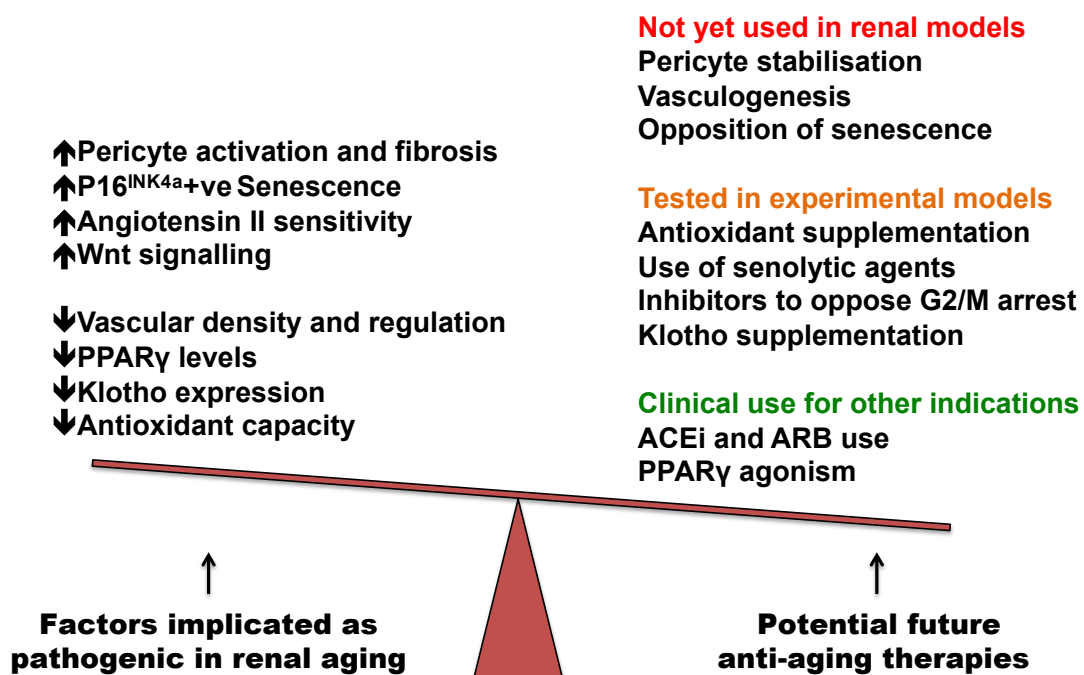
Multiple pathways interact to produce the changes of renal aging and ↓ GFR.

Black text indicates implicated upstream effectors of aging, whilst red text reports the functional and histological changes found in the aged kidney



## Figure 5 - Potential pathways and therapeutic targets for the treatment of renal aging

Proposed aging associated pathways (left side), and potential interventions to address these, coded by current use in patients (green), experimental use in models of renal disease/aging (orange) or potential for future study in the kidney (red).



**Table 1 - Studies of putative aging pathways *in vitro*, *in vivo* and in man**

Changes in activity of various signalling pathways and mechanisms implicated in the response of kidney to increasing age. Column 1 indicates cellular changes observed *in vitro*, column 2 reports effects seen in experimental models of renal aging and injury, and column 3 shows any reported effects in human aging and renal disease.

Aging factor	<i>In vitro</i> studies	Experimental studies	Human studies
<b>Telomere shortening</b>	Shown in cells to reduce with length of passage. Critical shortening leads to senescence <sup>106</sup> .	Reduced in mice with age <sup>108</sup> . Impaired regeneration after IRI <sup>109</sup>	Reduced with age, with oxidative stress, CKD and HD <sup>150,152</sup> . Risk factor for CVD <sup>151</sup> .
<b>Klotho signaling</b>	Klotho opposes signaling of IGF-1 and insulin <sup>43</sup> in cell lines in vitro.	Klotho deficiency decreases lifespan <sup>45</sup> . Overexpression reduces IGF-1 and Wnt signaling and increases lifespan <sup>43</sup>	Reduced with age <sup>132</sup> . Reduction associated with calcification and vascular disease <sup>136</sup>
<b>Wnt signaling</b>	Promotes profibrotic genes e.g. Snail, PAI1, MMP7 <sup>52</sup> .	Levels increase with injury and in response to falling Klotho with aging <sup>53</sup> . Mediates renal RAAS signalling <sup>58</sup>	Increases seen in CKD & linked to organ fibrosis <sup>197</sup>
<b>PPAR<math>\gamma</math> levels</b>	Reduces oxidative stress/senescence in human fibroblasts <sup>64</sup>	Reduced activity with age <sup>59,60</sup> . Agonists reduce renal inflammation/injury <sup>65</sup>	Studies of PPAR $\gamma$ agonists suggest reduction in rates of proteinuria in diabetics <sup>138</sup>
<b>Antioxidant capacity</b>		Aged rats have reduced renal antioxidant capacity, and enhanced renal injury <sup>79</sup> . Reduced oxidative stress lessens renal injury <sup>198</sup>	Higher levels of oxidative stress in human aging and in CKD <sup>74</sup> . AGE accumulates with age <sup>142</sup> .
<b>Fibrosis</b>	ATII promotes fibrosis of glomerular cells and promotes reduction of SIRT-3 <sup>90</sup>	Collagen I, III and TGF- $\beta$ upregulated in aging mice <sup>51</sup> and rats <sup>66</sup> . G2/M arrest is implicated in post injury renal fibrosis <sup>93</sup> .	Nephrosclerosis a feature of aging and of hypertensive renal disease <sup>11,12</sup> . Fibrosis and ATII hypersensitivity seen in aged kidneys <sup>141</sup>
<b>Senescence/ G1 Arrest</b>	Human and animal cells undergo senescence in vitro in response to stress or prolonged culture. <sup>95</sup> p16INK4a KO epithelial cells convert to mesenchyme more readily <sup>102</sup>	p16INK4a and SA-beta-gal are markers for senescent cells and increased in aged animals and post-injury. G2/M arrest seen in scarred kidneys in response to injury <sup>93</sup> .	Increased numbers of senescent renal cells correlate with increased injury and reduced transplant function <sup>146,147</sup> .
<b>Vascular changes</b>	Aged mice aortas have increased G2/M phase cell cycle arrest in vitro <sup>199</sup>	Reduced renal capillary density in aged mice <sup>125</sup> & in response to severe IRI <sup>115</sup>	Increased renal vascular tone and vascular stiffening with age <sup>200</sup> . Loss of efficacy of vasodilators <sup>201</sup>
<b>Pericyte behavior</b>	Pericytes (but not myofibroblasts) stabilize endothelial cell cultures in vitro <sup>174</sup>	Reduction of interstitial pericytes with aging <sup>125</sup> . Increased myofibroblasts in response to UUO and IRI injury <sup>202</sup>	Comparative studies in aged humans ( $\pm$ CKD) have not been undertaken